Please replace the second full paragraph on page 49 that precedes Table F with the following:

-- Preparation of non-endogenous known GPCRs is preferably accomplished by using TRANSFORMER SITE-DIRECTED™ Mutagenesis Kit (Stratagene, according to manufacturer's instructions) or QUIKCHANGE SITE-DIRECTED™ Mutagenesis (Clontech). Endogenous GPCR is preferably used as a template and two mutagenesis primers utilized, as well as, most preferably, a lysine mutagenesis oligonucleotide and a selection marker oligonucleotide (SEQ.ID.NO.: 252; included in Stratagene's kit). For convenience, the codon mutation incorporated into the known GPCR and the respective oligonucleotides are noted, in standard form (Table F): --

Please replace the paragraph bridging pages 71 and 72 with the following:

-- A less costly but equally applicable alternative has been identified which also meets the needs of large scale screening. FLASH PLATES™ and WALLAC™ scintistrips may be utilized to format a high throughput [^{35}S]GTPγS binding assay. Furthermore, using this technique, the assay can be utilized for known GPCRs to simultaneously monitor tritiated ligand binding to the receptor at the same time as monitoring the efficacy via [^{35}S]GTPγS binding. This is possible because the Wallac beta counter can switch energy windows to look at both tritium and ^{35}S-labeled probes. This assay may also be used to detect other types of membrane activation events resulting in receptor activation. For example, the assay may be used to monitor ^{32}P phosphorylation of a variety of receptors (both G protein coupled and tyrosine kinase receptors). When the membranes are centrifuged to the bottom of the well, the bound [^{35}S]GTPγS or the ^{32}P-phosphorylated receptor will activate the scintillant which is coated of the wells. SCINTI® strips (Wallac) have been used to demonstrate this principle. In addition, the assay also has utility for measuring ligand binding to receptors using radioactively labeled ligands. In a similar manner, when the radiolabeled bound ligand is centrifuged to the bottom of the well, the scintistrip label comes into proximity with the radiolabeled ligand resulting in activation and detection. --

Please replace the paragraph on page 72 that begins with "A Flash Plate" and ends with "express the receptors" with the following:

B5

-- A FLASH PLATE™ Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) designed for cell-based assays can be modified for use with crude plasma membranes. The Flash Plate wells contain a scintillant coating which also contains a specific antibody recognizing cAMP. The cAMP generated in the wells was quantitated by a direct competition for binding of radioactive cAMP tracer to the cAMP antibody. The following serves as a brief protocol for the measurement of changes in cAMP levels in membranes that express the receptors. --

Please replace the paragraph bridging pages 72 and 73 with the following:

B6

-- Transfected cells are harvested approximately three days after transfection. Membranes were prepared by homogenization of suspended cells in buffer containing 20mM HEPES, pH 7.4 and 10mM $MgCl_2$. Homogenization is performed on ice using a Brinkman POLYTRON™ for approximately 10 seconds. The resulting homogenate is centrifuged at 49,000 X g for 15 minutes at 4°C. The resulting pellet is then resuspended in buffer containing 20mM HEPES, pH 7.4 and 0.1 mM EDTA, homogenized for 10 seconds, followed by centrifugation at 49,000 X g for 15 minutes at 4°C. The resulting pellet can be stored at -80°C until utilized. On the day of measurement, the membrane pellet is slowly thawed at room temperature, resuspended in buffer containing 20mM HEPES, pH 7.4 and 10mM $MgCL_2$ (these amounts can be optimized, although the values listed herein are preferred), to yield a final protein concentration of 0.60mg/ml (the resuspended membranes were placed on ice until use). --

Please replace the paragraph on page 73 that begins with "cAMP standards and" and ends with "assay plate" with the following:

B7

-- cAMP standards and Detection Buffer (comprising 2 μCi of tracer [^{125}I cAMP (100 μl)] to 11 ml Detection Buffer) are prepared and maintained in accordance with the manufacturer's instructions. Assay Buffer is prepared fresh for screening and contained 20mM HEPES, pH 7.4, 10mM $MgCl_2$, 20mM (Sigma), 0.1 units/ml creatine phosphokinase (Sigma), 50 μM GTP (Sigma), and 0.2 mM ATP (Sigma); Assay Buffer

can be stored on ice until utilized. The assay is initiated by the addition of 50μL of assay buffer followed by addition of 50 μL of membrane suspension to the NEN Flash Plate. The resultant assay mixture is incubated for 60 minutes at room temperature followed by addition of 100 μL of detection buffer. Plates are then incubated an additional 2-4 hours followed by counting in a Wallac MICROBETA™ scintillation counter. Values of cAMP/well are extrapolated from a standard cAMP curve that is contained within each assay plate. --

Please replace the paragraph bridging pages 74 and 75 with the following:

-- A FLASH PLATE™ Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) designed for cell-based assays can be modified for use with crude plasma membranes. The Flash Plate wells can contain a scintillant coating which also contains a specific antibody recognizing cAMP. The cAMP generated in the wells can be quantitated by a direct competition for binding of radioactive cAMP tracer to the cAMP antibody. The following serves as a brief protocol for the measurement of changes in cAMP levels in whole cells that express the receptors. --

Please replace the paragraph bridging pages 77 and 78 with the following:

-- A method to detect Gs stimulation depends on the known property of the transcription factor CREB, which is activated in a cAMP-dependent manner. A PATHDETECT™ CREB trans-Reporting System (Stratagene, Catalogue # 219010) can utilized to assay for Gs coupled activity in 293 or 293T cells. Cells are transfected with the plasmids components of this above system and the indicated expression plasmid encoding endogenous or mutant receptor using a Mammalian Transfection Kit (Stratagene, Catalogue #200285) according to the manufacturer's instructions. Briefly, 400 ng pFR-Luc (luciferase reporter plasmid containing Gal4 recognition sequences), 40 ng pFA2-CREB (Gal4-CREB fusion protein containing the Gal4 DNA-binding domain), 80 ng pCMV-receptor expression plasmid (comprising the receptor) and 20 ng CMV-SEAP (secreted alkaline phosphatase expression plasmid; alkaline phosphatase activity is measured in the media of transfected cells to control for variations in transfection efficiency between samples) are combined in a calcium phosphate precipitate per the

Kit's instructions. Half of the precipitate is equally distributed over 3 wells in a 96-well plate, kept on the cells overnight, and replaced with fresh medium the following morning. Forty-eight (48) hr after the start of the transfection, cells are treated and assayed for, e.g., luciferase activity. --

Please replace the paragraph bridging pages 80 and 81 with the following:

-- A method to detect Gq stimulation depends on the known property of Gq-dependent phospholipase C to cause the activation of genes containing AP1 elements in their promoter. A PATHDETECT™ AP-1 cis-Reporting System (Stratagene, Catalog # 219073) can be utilized following the protocol set forth above with respect to the CREB reporter assay, except that the components of the calcium phosphate precipitate were 410 ng pAP1-Luc, 80 ng pCMV-receptor expression plasmid, and 20 ng CMV-SEAP. –

Please replace the paragraph on page 81 that is between headings 4 and 5 with the following:

-- One method to detect Gq stimulation depends on the known property of Gq-dependent phospholipase C to cause the activation of genes containing serum response factors in their promoter. A PATHDETECT™ SRF-Luc-Reporting System (Stratagene) can be utilized to assay for Gq coupled activity in, e.g., COS7 cells. Cells are transfected with the plasmid components of the system and the indicated expression plasmid encoding endogenous or non-endogenous GPCR using a MAMMALIAN TRANSFECTION™ Kit (Stratagene, Catalogue #200285) according to the manufacturer's instructions. Briefly, 410 ng SRF-Luc, 80 ng pCMV-receptor expression plasmid and 20 ng CMV-SEAP (secreted alkaline phosphatase expression plasmid; alkaline phosphatase activity is measured in the media of transfected cells to control for variations in transfection efficiency between samples) are combined in a calcium phosphate precipitate as per the manufacturer's instructions. Half of the precipitate is equally distributed over 3 wells in a 96-well plate, kept on the cells in a serum free media for 24 hours. The last 5 hours the cells are incubated with 1mM Angiotensin, where indicated. Cells are then lysed and assayed for luciferase activity using a LUCLITE™ Kit (Packard, Cat. # 6016911) and "Trilux 1450 Microbeta" liquid scintillation and

luminescence counter (Wallac) per the manufacturer's instructions. The data can be analyzed using GRAPHPAD PRISM™ 2.0a (GraphPad Software Inc.). --

Please replace the second paragraph under Example 7 on page 91 with the following:

-- A modified FLASH PLATE™ Adenylyl Cyclase kit (New England Nuclear; Cat. No. SMP004A) is preferably utilized for confirmation of candidate compounds directly identified as inverse agonists and agonists to non-endogenous, constitutively activated GPCR in accordance with the following protocol. –

Please replace the paragraph bridging pages 91 and 92 with the following:

-- Transfected cells will be harvested approximately three days after transfection. Membranes are prepared by homogenization of suspended cells in buffer containing 20mM HEPES, pH 7.4 and 10mM $MgCl_2$. Homogenization is performed on ice using a Brinkman POLYTRON™ for approximately 10 seconds. The resulting homogenate will be centrifuged at 49,000 X g for 15 minutes at 4°C. The resulting pellet is then resuspended in buffer containing 20mM HEPES, pH 7.4 and 0.1 mM EDTA, homogenized for 10 seconds, followed by centrifugation at 49,000 X g for 15 minutes at 4°C. The resulting pellet can be stored at -80°C until utilized. On the day of direct identification screening, the membrane pellet is slowly thawed at room temperature, resuspended in buffer containing 20mM HEPES, pH 7.4 and 10mM $MgCl_2$, to yield a final protein concentration of 0.60mg/ml (the resuspended membranes are placed on ice until use). --

Please replace the last paragraph on page 93 with the following:

-- Following the incubation, 100µl of Detection Buffer is added to each well, followed by incubation for 2-24 hours. Plates are then counted in a Wallac MICROBETA™ plate reader using "Prot. #31" (as per manufacturer instructions). --